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Proliferating Cell Nuclear Antigen Expression in the Gallbladder With Pancreaticobiliary Maljunction

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Background and Objective: To clarify the histogenesis of cancer of the gallbladder of the patients with pancreaticobiliary maljunction (PBMJ), the proliferating cell activity of the epithelium of gallbladder was examined.
Methods: A total of 21 patients with pancreaticobiliary maljunction [8 with gallbladder cancer (MCA group) and 13 without cancer (M group)] were studied using immunohistochemical staining with a monoclonal antibody to the proliferating cell nuclear antigen (PCNA). The 23 gallbladder cancer patients without PBMJ (CA group) and 10 patients with normal gallbladders (N group) were also examined as controls.
Results: The PCNA-positive rates of non-cancerous epithelium of the gallbladder in patients with PBMJ (14.2% in MCA group and 11.6% in M group) were significantly higher than those without PBMJ (3.9% in CA group and 1.5% in N group).
Conclusion: The high proliferating cell activity of epithelium of the gallbladder may be an explanation for the high incidence of gallbladder cancer in patients with pancreaticobiliary maljunction.
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KEY WORDS: cancer of the gallbladder; proliferating cell activity; anomalous arrangement of the pancreaticobiliary ductal system

INTRODUCTION

Pancreaticobiliary maljunction (PBMJ), an anomalous arrangement of the pancreaticobiliary ductal system, is a congenital anomaly defined as a union of the pancreatic and biliary ducts that is located outside of the duodenal wall, and PBMJ is commonly observed in patients with congenital choledochal cysts [1,2]. It is well known that patients with PBMJ frequently develop cancer of the biliary tract, and its incidence is reported to be 23–37% in the Japanese literature [3]. However, the carcinogenesis of gallbladder cancer in patients with PBMJ remains unclear, although several studies have been performed on the subject. In the present study, to clarify one of the possible carcinogenic factors, we examined cell proliferating activity in the epithelium of the gallbladder with PBMJ using immunohistochemical expression of proliferating cell nuclear antigen (PCNA) and compared it with that of cases without PBMJ.

PATIENTS AND METHODS

A total of 21 gallbladders of patients with PBMJ [8 patients with cancer of the gallbladder (MCA group) and 13 patients without cancer (M group)] were studied using immunohistochemical staining with a monoclonal antibody to the PCNA. In all these patients, PBMJ was preoperatively diagnosed by endoscopic retrograde cholangiopancreatography (ERCP).

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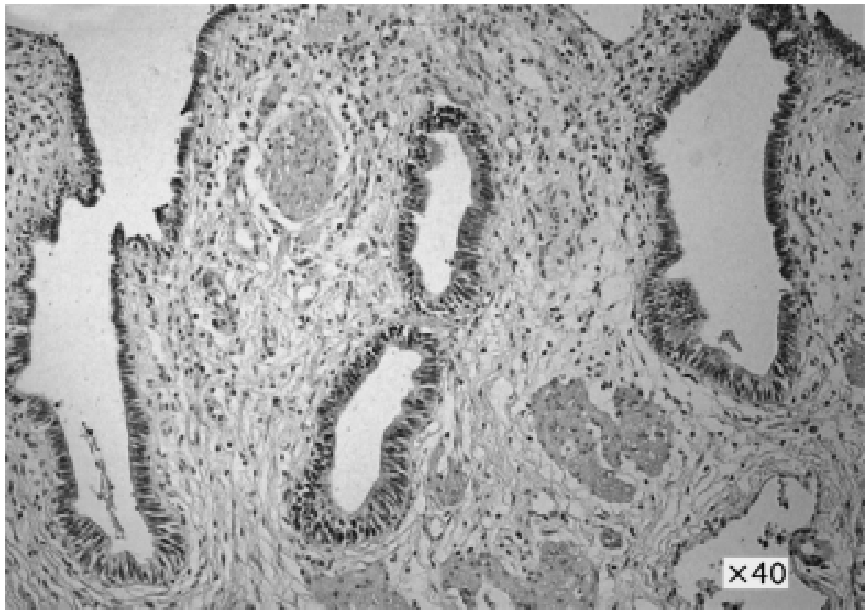

Fig. 1. Immunohistochemical staining of noncancerous epithelium of the gallbladder with pancreaticobiliary maljunction. The anti-proliferating cell nuclear antigen (PCNA) monoclonal antibody positively stains the epithelial cell nuclei brown, with a PCNA positive rate of 54.2%. (Original magnification ×40.)

In addition, 23 gallbladders of the gallbladder cancer patients without PBMJ (CA group) and 10 normal gallbladders of the gastric cancer patients which were resected during gastric surgery (N group) were also examined as controls. In the CA group, concomitant gallstones were observed in 16 of 23 patients. One patient in the MCA group had concomitant bile duct cancer.

Age and sex of the groups were 59.0 ± 7.6 (mean ± SD) in the MCA group (8 females), 42.2 ± 15.1 in the M group (3 males and 10 females), 64.4 ± 10.4 in the CA group (5 males and 18 females), and 66.8 ± 8.4 in the N group (5 males and 5 females).

Concerning the bile duct dilatation which frequently accompanies PBMJ, there were 5 patients with bile duct dilatation and 3 patients without it in the MCA group, and 11 patients with bile duct dilatation and 2 patients without it in the M group.

Immunohistochemical Staining by the Indirect Enzyme-Labeled Antibody Method

Tissue sections, 4 μm in thickness, were prepared from a block taken from the central part of the gallbladder, and from cancerous lesions if present. These sections were embedded in paraffin following fixation in 10% formalin for 2–5 days, then dewaxed in xylene and rehydrated through alcohol, after endogenous peroxidase was inhibited, and normal sheep serum applied for 30 min to reduce nonspecific antibody bindings. Subsequently, the sections were incubated overnight at 4°C with the antiproliferating cell nuclear antigen (PCNA) monoclonal antibody (PC 10, Dako A/S, Copenhagen, Denmark) diluted to 1:50 as the primary antibody. They were then incubated with HRP-labeled antimouse Ig antibody (Amersham, Buckinghamshire, U.K.) as the secondary antibody at room temperature for 1 hr, followed by color development with diaminobenzidine.

Evaluation of PCNA Expression

Assessment of PCNA expression was carried out by one examiner who was not aware of the clinopathological profile. The number of positive cells for every 500 cells was calculated twice and the mean value expressed as the PCNA-positive rate (Fig. 1).

The statistical significance of differences was evaluated by Wilcoxon-Mann-Whitney test, in which two items were compared, and a P value of < 0.05 was considered significant.

RESULTS

The PCNA-Positive Rates of Noncancerous Epithelium of the Gallbladder

The PCNA-positive rates of noncancerous epithelium of the gallbladder in patients with PBMJ (14.2% in the MCA group and 11.6% in the M group) were significantly higher than those without maljunction (3.9% in CA group and 1.5% in N group, Fig. 2).

The PCNA-Positive Rates in Cancerous Lesion

The PCNA-positive rate in cancerous lesions of the MCA group (33.0%) was similar to that of the CA group

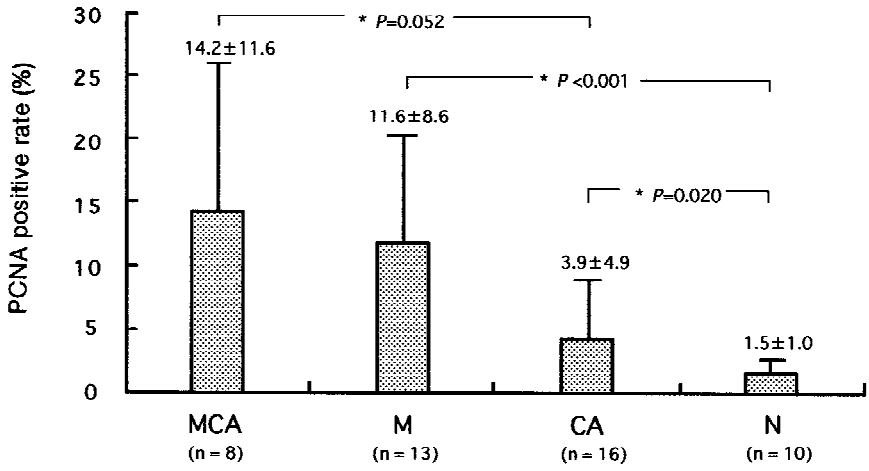

Fig. 2. Proliferating cell nuclear antigen (PCNA) positive rate in noncancerous epithelium of the gallbladder. MCA, pancreaticobiliary maljunction (PBMJ) with cancer of the gallbladder; M, PBMJ without cancer of the gallbladder; CA, cancer of the gallbladder without PBMJ; N, normal gallbladder of gastric cancer patients; *significant by Wilcoxon-Mann-Whitney test.

(31.6%, Fig. 3). In patients with PBMJ, there was no relationship between the PCNA-positive rate of noncancerous epithelium and age or amylase level in bile juice in gallbladder, although the amylase level in bile juice was over 18,500 U/L in 9 of 14 patients

DISCUSSION

PBMJ, especially in patients without dilatation of the bile duct, is frequently complicated by gallbladder cancer. Carcinogenesis in PBMJ has been explained by the fact that pancreatic enzymes regurgitated into the bile duct were activated and concentrated in the gallbladder, which irritates the epithelium [4,5]. The changes of bile acid in PBMJ were also considered one of the carcinogenic factors [6]. Histological studies [7,8] of the gallbladder with PBMJ frequently demonstrated severe dysplastic epithelium in mucosa adjacent to the tumor, and in our series, five of eight gallbladder cancer patients with PBMJ had dysplastic epithelium in the noncancerous mucosa of the gallbladder (data not shown).

Another aspect of understanding carcinogenesis of gallbladder cancer with PBMJ is the cell kinetics in biliary epithelium. Noguchi [9] studied the cell kinetics of the gallbladder epithelium with PBMJ, using the BrdU labeling index (BrdU LI), in comparison with those of the epithelium of normal gallbladder, cholelithiasis, and the primary cancerous lesion of gallbladder cancer without PBMJ, and reported that the BrdU LI of noncancerous gallbladder epithelium with PBMJ was as high as that of the cancerous lesion itself. Fujita et al. [10] also performed cell kinetic studies similar to Noguchi's study using BrdU LI. They compared the BrdU LI values in noncancerous gallbladder epithelium of group I (no biliary disorder group), group II (cholecystolithiasis), group III (PBMJ), group IV (gallbladder cancer with PBMJ),

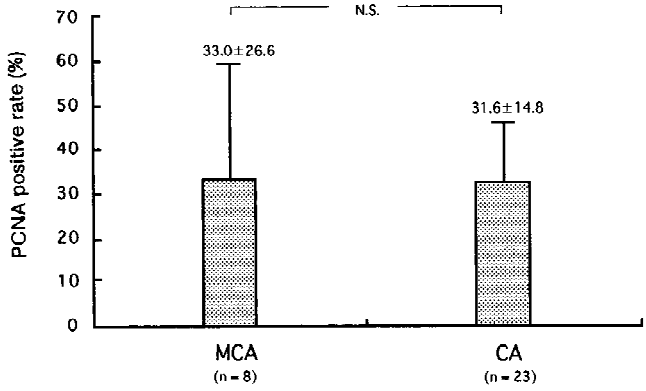

Fig. 3. Proliferating cell nuclear antigen (PCNA) positive rate in cancerous lesions of the gallbladder. MCA, pancreaticobiliary maljunction (PBMJ) with cancer of the gallbladder; CA, cancer of the gallbladder without PBMJ; N.S., not significant by Wilcoxon-Mann-Whitney test.

and group V (gallbladder cancer with cholecystolithiasis without PBMJ), and demonstrated that the BrdU LI of groups II, III, IV, and V were significantly higher than that of group I, whereas there were no significant differences among groups II, III, IV, and V. However, in their study, the numbers examined in each group were relatively small.

In the present study, we used proliferating cell nuclear antigen staining (PCNA-positive rate) as an indicator of cell proliferating activity [11]. One of the greatest advantages of PCNA immunohistochemical staining is that formalin-fixed and paraffin-embedded materials can be used for its assessment, whereas the BrdU method requires raw materials.

In this series, the non-cancerous gallbladder epitheliums with PBMJ, regardless of concomitant cancer, were shown to have significantly higher PCNA-positive rates than those without PBMJ. High cell proliferating activity

itself is not a carcinogenic factor, but it indicates a possibility of malignant change. In fact, the PCNA-positive rates in cancerous lesions were over 30%. Therefore, these results present theoretical bases for a recommendation of biliary reconstruction in younger patients with PBMJ.

On the other hand, although the PCNA-positive rate in noncancerous gallbladder epithelium of gallbladder cancer without PBMJ (CA group) was significantly higher than that of normal gallbladder, it is very low compared with those of gallbladder with PBMJ. In addition, there was no significant difference between the PCNA-positive rate of noncancerous epithelium in the CA group with gallstones (2.7%, n = 4) and in those without (4.3%, n = 12). We did not examine cholecystolithiasis alone in this series. However, in Noguchi's report, the BrdU LI of gallbladder epithelium of cholecystolithiasis alone was significantly lower than that of gallbladder with PBMJ, and the BrdU LI of noncancerous epithelium of gallbladder cancer without PBMJ was also low. Those results agree with those of the present study. Therefore, it was supposed that the mechanism of carcinogenesis of gallbladder cancer with and without PBMJ would be different.

We did not present the data of the PCNA-positive rate in the epithelium of the bile duct in this series, because in most cases with PBMJ the epithelium of the bile duct had a tendency to be denuded, especially in the patients with dilatation of the bile duct. However, reflux of the pancreatic juice into the bile duct also causes bile duct carcinoma, frequently in patients with dilatation of the bile duct, and even in patients with nondilated bile duct. Therefore, excision of the entire extrahepatic bile duct and biliary reconstruction is thought to be necessary for patients with PBMJ [3,12].

In conclusion, the proliferating cell activity of epithelium of the gallbladder was higher in patients with PBMJ than in patients without. This may be one of the explanations of the high incidence of gallbladder cancer in patients with pancreaticobiliary maljunction. Thus, for patients with PBMJ, early excision of the gallbladder and bile duct with biliary reconstruction should be recommended regardless of concomitant biliary cancer.

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